A Physically Anchored Genetic Map and Linkage to Avirulence Reveals Recombination Suppression Over the Proximal Region of Hessian Fly Chromosome A2

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ABSTRACT

Resistance in wheat (*Triticum aestivum*) to the Hessian fly (*Mayetiola destructor*), a major insect pest of wheat, is based on a gene-for-gene interaction. Close linkage (3 ± 2 cM) was discovered between Hessian fly avirulence genes *vH3* and *vH5*. Bulked segregant analysis revealed two DNA markers (28-178 and 23-201) within 10 cM of these loci and only 3 ± 2 cM apart. However, 28-178 was located in the middle of the short arm of Hessian fly chromosome A2 whereas 23-201 was located in the middle of the long arm of chromosome A2, suggesting the presence of severe recombination suppression over its proximal region. To further test that possibility, an AFLP-based genetic map of the Hessian fly genome was constructed. Fluorescence *in situ* hybridization of 20 markers on the genetic map to the polytene chromosomes of the Hessian fly indicated good correspondence between the linkage groups and the four Hessian fly chromosomes. The physically anchored genetic map is the first of any gall midge species. The proximal region of mitotic chromosome A2 makes up 30% of its length but corresponded to <3% of the chromosome A2 genetic map.

THE Hessian fly, *Mayetiola destructor*, is a destructive pest of wheat, *Triticum aestivum*. Present in most parts of the world, it is often managed by planting Hessian-fly-resistant cultivars (Hatchett *et al.* 1987). Use of these cultivars has selected for "virulent" Hessian fly genotypes that are able to overcome cultivar-specific resistance (Gallun 1977; Ratcliffe *et al.* 1994). Therefore, strains (biotypes) of the various virulent and avirulent Hessian fly genotypes have been propagated for decades in the greenhouse to facilitate the discovery of new resistance genes (Cartwright and LaHue 1944; Gallun *et al.* 1961; Hatchett *et al.* 1987). By investigating the inheritance of virulence among these biotypes, Hatchett and Gallun (1970) discovered that the Hessian fly and wheat have a gene-for-gene relationship (Flor 1956).

To date, 30 different Hessian fly resistance genes have been discovered in wheat and in its close relatives (Martin-Sanchez *et al.* 2003). Except for resistance genes *H7* and *H8*, which must be combined to provide effective resistance (Patterson and Gallun 1973), each resistance gene is simply inherited and, except for *h4*, resistance alleles at each locus are dominant or semidominant to susceptibility alleles (Ratcliffe and Hatchett 1997). Virulence to most of these genes has been observed in Hessian fly populations in the United States (Ratcliffe *et al.* 1997) and in the Middle East (El Bouhssini *et al.* 1998; Naber *et al.* 2000), but purification and analysis of these genotypes have been limited. Virulence and avirulence to resistance genes *H3*, *H5*, *H6*, *H7H8*, *H9*, and *H13* is clearly conditioned by simply inherited recessive alleles of distinct loci in the Hessian fly (Harris *et al.* 2003). These loci have been named *vH3*, *vH5*, *vH6*, *vH7H8*, *vH9*, and *vH13* according to the nomenclature established by Formusoh *et al.* (1996). Avirulence genes *vH6*, *vH9*, and *vH13* are X-linked and *vH13* has been positioned within a 10-cM region flanked by molecular markers (Rider *et al.* 2002). Avirulence genes *vH3*, *vH5*, and *vH7H8* are autosomal (Gallun 1978; Harris *et al.* 2003), but have not yet been mapped.

To perform genetic analyses in the Hessian fly it was important to understand its anomalous chromosome cycle and method of sex determination (Stuart and Hatchett 1988a,b, 1991; Shukle and Stuart 1993). Chromosome imprinting is apparent during both gametogenesis and embryogenesis. The germ line of every Hessian fly contains two sets of chromosomes, a germline-limited set called the E chromosomes and a set that is found in both the germ line and the soma, the S

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chromosomes. The S chromosomes are composed of two autosomes (A1 and A2) and two X chromosomes (X1 and X2). The S chromosomes undergo recombination during oogenesis and the resulting ova contain a haploid set of S chromosomes and 30–40 E chromosomes (A1 A2 X1 X2 + E). There is no recombination during spermatogenesis and the resulting sperm contain only the maternally derived S chromosomes (A1 A2 X1 X2). Thus, just after the sperm and ova combine, each zygote contains a diploid set of S chromosomes and 30–40 E chromosomes (A1 A2 X1 X2/A1 A2 X1 X2 + E). The E chromosomes are eliminated from the presumptive somatic nuclei during the fifth nuclear division of embryogenesis (Bantock 1970). During that division, the paternally derived X1 and X2 chromosomes may also be eliminated from the presumptive somatic nuclei. If they are retained, the somatic cells have a female karyotype (A1 A2 X1 X2/A1 A2 X1 X2). If they are eliminated, the somatic cells have a male karyotype (A1 A2 X1 X2/A1 A2 O O). The elimination or maintenance of the paternally derived X chromosomes is controlled by maternal genotype. Thus, most females produce either all-female or all-male offspring.

In this study, our objective was to genetically map autosomal avirulence loci *vH3*, *vH5*, and *vH7H8*. We modified a bulked segregant analysis approach used to map X-linked avirulence genes (Stuart *et al.* 1998) to accommodate autosomal loci and identified two molecular markers genetically linked to both *vH3* and *vH5*. The chromosomal positions of these markers suggested that the avirulence loci are on chromosome A2 and that severe recombination suppression exists over the proximal region of that chromosome. This was tested with two additional mapping populations and by constructing the first genetic map of the Hessian fly S chromosomes. By determining the polytene chromosomal positions of amplified fragment length polymorphism (AFLP) and sequence-tagged site (STS) markers on the genetic map, a useful physically anchored genetic map of the Hessian fly S genome was developed.

MATERIALS AND METHODS

Hessian fly strains and experimental matings: Hessian fly strains were maintained as families of individual females on separate caged pots (10-cm diameter) of wheat seedlings as previously described (Rider *et al.* 2002). Mated female Hessian flies generally produced 50–200 eggs and deposited these between the veins on the upper surface of the leaves of 20–30 wheat seedlings grown in each pot. The life cycle was completed in 25–33 days at 20° ± 2° on susceptible seedlings. Hessian fly resistance in wheat was scored as the manifestation of normal plant growth and the death of first instar larvae. Hessian fly virulence was scored as the manifestation of stunted plant growth and normal development of Hessian fly larvae.

Four Hessian fly strains were used in this investigation. The "Great Plains biotype" (GP) was originally collected in Kansas and maintained in the greenhouse for 5 years. GP flies are avirulent to all known resistance genes. The "biotype-L" (L) strain was originally collected in Indiana and has been maintained in the greenhouse at Purdue University for over 20 years. L flies are virulent to wheat resistance genes *H3*, *H5*, *H6*, and *H7H8* and avirulent to all other known resistance genes. The "*vH13*" population was derived from a "biotype-E" population originally collected in Georgia and is virulent to Hessian fly resistance genes *H3* and *H13*, but avirulent to *H5* (Rider *et al.* 2002). The Indiana population is virulent to Hessian fly resistance genes *H6* and *H9*. All Hessian fly populations and matings were maintained at 20° ± 2° under a 12:12 photoperiod in environmental chambers at Purdue University.

Virulent and avirulent phenotypes of individual females from both the L and the GP populations were checked with respect to resistance genes *H3*, *H5*, and *H7H8* for two generations. Crosses were then made between L females and GP males derived from these "purified" populations (Figure 1). F_1 females produced by these matings were then backcrossed to GP males to produce male BC_1 families. Two such families were selected to develop two mapping populations. Both mapping populations were developed using procedures described previously (Rider *et al.* 2002) with modifications to accommodate autosomal avirulence genes. Mapping population 1 was used for both recombination analysis and bulked segregant analysis. Mapping population 2 was used only for recombination analysis. Male Hessian flies transmit only their maternally derived chromosomes to their offspring (Stuart and Hatchett 1988a). Therefore, each BC_1 male was testcrossed separately to an L female and the virulent and avirulent phenotypes of each BC_1 male were determined by scoring the ability of their testcross offspring to live on the resistant wheat seedlings in the pot. The genotypes of population 1 BC_1 males were determined with respect to virulence and avirulence to *H3*, *H5*, and *H7H8* by caging the mated females on pots containing wheat seedlings of "Monon" (*H3*), "Abe" (*H5*), "Seneca" (*H7H8*), and "Blueboy" (susceptible check). The genotypes of population 2 BC_1 males were determined with respect to only *H5* by caging the mated females on pots containing seedlings of "Abe" and "Blueboy." After the testcross mating, each BC_1 male was collected separately for DNA extraction as described below.

A third mapping population was used to develop an AFLP-based genetic map of the Hessian fly genome. This population was initiated by a mating between a *vH13* female and an Indiana male. A single F_1 female produced by this mating was then backcrossed to a *vH13* male and 55 BC_1 females produced by this mating were separately collected for DNA extraction to construct the AFLP-based genetic map.

AFLP-PCR: AFLP-PCR was performed using the AFLP system for small genomes (Invitrogen, San Diego) and ^{33}P-end-labeled selective primers. All components in the reactions were scaled to one-half the recommended volume and quantity. Reaction products were separated by electrophoresis through 6% denaturing LongRanger (Cambrex) polyacrylamide gels. The gels were dried and exposed to Biomax MR X-ray film (Eastman-Kodak, Rochester, NY) for autoradiography. To name the AFLPs identified in these experiments, the following methodology was adopted: The selective *Eco*RI primers were numbered 1–8 according to the two unique bases at their 3′-ends (AA, 1; AC, 2; AG, 3; AT, 4; TA, 5; TC, 6; TG, 7; and TT, 8). Likewise, the selective *Mse*I primers were numbered 1–8 according to the three unique bases at their 3′-ends (CAA, 1; CAC, 2; CAG, 3; CAT, 4; CTA, 5; CTC, 6; CTG, 7; and CTT, 8). Each polymorphic DNA fragment was given a unique name by using the *Eco*RI selective primer number followed by the *Mse*I selective primer number followed by either a dash and the size of the DNA fragment in base pairs or a two-digit number. In the latter case, polymorphisms with

the highest relative molecular weight were numbered "01," and the numbers progressively increased as the relative size of the polymorphisms decreased.

Bulked segregant analysis and linkage analysis: To identify AFLPs linked to the avirulence genes *vH5* and *vH3*, bulked segregant analysis (Michelmore *et al.* 1991) was performed with modifications to accommodate autosomal Hessian fly avirulence genes (Figure 1). Genomic DNA was isolated from individual BC_1 males using a DNeasy tissue kit (QIAGEN, Chatsworth, CA). Equal amounts of DNA (20 ng) from each of 15 *H3*- and *H5*-virulent and 15 *H3*- and *H5*-avirulent BC_1 male flies were pooled separately to prepare bulk DNA corresponding to the two phenotypes. Each pool of DNA was then prepared for AFLP-PCR. A total of 64 *Eco*RI and *Mse*I selective primer combinations were used to generate DNA fingerprints of the two DNA pools.

STS markers were developed on the basis of sequences of AFLPs and Hessian fly genomic λ-clones as described below. Individual Hessian flies from each mapping population were genotyped for these markers to determine their genetic positions relative to avirulence genes *vH5* and *vH3* and other markers on the AFLP-based genetic map of the Hessian fly genome. This was performed by PCR using genomic DNA of each male and the STS primers designed as described below.

To generate an AFLP-based genetic map of the Hessian fly genome, AFLP-PCR was performed on DNA derived from individual females collected from a small ($N = 55$) Hessian fly family. DNA from each female was prepared as described above. AFLP-PCR was performed using 16 selective *Eco*RI and *Mse*I primer combinations. Segregating AFLP loci were tested for significant deviation from the expected 1:1 Mendelian ratio ($\chi^2 = 3.84$, d.f. $= 1$, $\alpha = 0.05$). Multipoint linkage analysis was performed with MAPMAKER 3.0b (Lander *et al.* 1987; Lincoln *et al.* 1992) using the Kosambi centimorgan function. Linkage groups were initially declared using a minimum LOD score of 4.0 and a maximum distance of 21 cM. Preliminary map orders were generated using the "order" command with a minimum LOD of 3.0 and up to 25 randomly chosen subsets. The preliminary linkage maps defined in this way were retained and other markers were added if they did not significantly alter the map (map expansion <4 cM). When several markers cosegregated, one marker was chosen to represent the locus.

Development of STS markers: For bulked segregant analysis, AFLPs linked to avirulence were expected in association with only the avirulent DNA pool (Figure 1). To convert these polymorphisms into site-specific STS markers, all (eight) of the polymorphic bands that fit that pattern were extracted from the gels and cloned into pCR4-TOPO Vector using a TOPO TA cloning kit (Invitrogen). Eight clones of each transformation were then sequenced in both directions using an ABI 3700 sequencer in the Purdue University Core Genomics Facility. Site-specific primers were designed for PCR on the basis of those sequences (Figure 2).

Additional STS markers were developed on the basis of sequences of Hessian fly genomic λ-clones previously positioned on the Hessian fly polytene chromosomes by *in situ* hybridization (Shukle and Stuart 1995). To obtain sequence from each λ-clone, each was digested to completion with *Eco*RI and the fragments were subcloned into the plasmid vector pGEM-7 (Promega, Madison, WI). Subclones were then selected for sequencing as described above, and site-specific primers were designed for PCR on the basis of that sequence (Figure 2).

PCR was performed in 25-μl reaction volumes containing 10 mm Tris-Cl (pH 8.0), 50 mm KCl, 1.5 mm $MgCl_2$, 0.01% gelatin, 200 μm each of the four dNTPs, 200 nm of each primer, 30 ng of genomic DNA, and 2.5 units of *Taq* DNA polymerase. The PCR products were separated by electrophoresis in 2% agarose gels and visualized by ethidium bromide staining. The lower and higher alleles amplified by each polymorphic marker were then cloned separately and their nucleotide sequences were determined as described above to identify allelic differences.

Linkage group-chromosome correlations: To establish correlations between AFLP-based genetic linkage groups and the polytene chromosomes of the Hessian fly, cloned AFLP bands and STS markers were used as probes to identify clones in an arrayed Hessian fly bacterial artificial chromosome (BAC) library. The BAC clone DNA was then used as probe in fluorescence *in situ* hybridization (FISH) experiments to determine their positions on the Hessian fly polytene chromosomes. To obtain DNA sequence of AFLP bands representing loci on the genetic map, AFLPs were extracted from acrylamide gels, amplified using the same selective AFLP primers, and cloned into the pCR4TOPO vector as described above. The DNA sequences of seven or eight clones from each transformation were then sequenced separately and site-specific primers for PCR were designed for each AFLP on the basis of that sequence. When more than one sequence was associated with a single AFLP band, primers were designed on the basis of only one sequence, which corresponded in size with that of the AFLP band taken from the gel plus the selective AFLP primers that were used to produce the AFLP. The primers were used in PCR to amplify the cloned AFLP fragments and the cloned STS fragments. The resulting amplicons were then used as template to produce ^{32}P-labeled probes in separate random priming reactions using the DNA labeling system (Invitrogen) according to the manufacturer's recommendations.

To prepare the Hessian fly BAC library, Hessian fly nuclei were isolated in 1% agarose plugs at Purdue University. The plugs were then shipped to Research Genetics (Invitrogen, Carlsbad, CA) where they were partially digested with *Hin*dIII and the resulting DNA fragments were then cloned into the vector pBeloBAC-Indigo. Recombinant molecules were then transformed into *Escherichia coli* and the library was shipped to Purdue as a glycerol stock. The library was then plated and 18,432 clones were arrayed in 48 384-well plates at the Purdue Genomics Center. A sample of 20 clones arbitrarily selected from the library indicated that the average insert size was 55 ± 20 kb (data not shown). Nylon filters of the BAC library were prepared in the Purdue Genomics Center with a Qpix robot (Genetix). The filters were prehybridized for 4 hr at 60° in hybridization solution (10× Denhardt's, 6× SSC, 50 mm NaH_2PO_4, 10 mg/ml herring sperm DNA, and 0.5% SDS). They were hybridized at 60° for 16 hr in hybridization solution containing 10% polyethylene glycol and denatured probe. Membranes were exposed to Biomax MR (Kodak) film for 48 hr for autoradiography. DNA from positive BAC clones was isolated using a PSI clone BAC DNA kit (Princeton Separations) according to the manufacturer's recommendations. Each positive BAC clone was tested for the presence of the corresponding AFLP or STS sequences by PCR and prepared for FISH as described below.

FISH: Isolation of polytene chromosome from the salivary glands of second instar Hessian fly larvae and slide preparation were performed as previously described (Shukle and Stuart 1995). Probes were prepared by labeling BAC clone DNA (~1 μg) with either biotin- or digoxigenin-conjugated dUTP (Roche) by nick translation. Hybridizations were performed with 40–100 ng of denatured probe DNA in 10 μl of hybridization solution (10% dextran solution, 2× SSC, 40% formaldehyde, and 20 μg of herring sperm DNA) at 37° for 12–15 hr. Detection was performed using Alexa Fluor (Molecular Probes, Eugene, OR)-conjugated antibiotin and rhodamin-

TABLE 1

Numbers of virulent and avirulent BC_1 male phenotypes to Hessian fly resistance genes *H3*, *H5*, and *H7H8* present in two [(L × GP) × GP] Hessian fly mapping populations

	H5			*H3*			*H7H8*			
Population	Vir	Avr	χ^2	Vir	Avr	χ^2	Vir	Avr	χ^2	Total
1	31	37	0.5–0.25	30	38	0.5–0.25	62	6	>0.005	68
2	44	58	0.5–0.25	—	—	—	—	—	—	102

Vir, virulent; Avr, avirulent.

conjugated antidigoxigenin. Digital images were taken under UV optics using an ORCA-ER (Hamamatsu, Bridgewater, NJ) digital camera mounted on an Olympus BX51 microscope and MetaMorph (Universal Imaging, West Chester, PA) imaging software.

RESULTS

Genetic linkage between *vH3* and *vH5*: Mapping population 1 was used in an attempt to determine the genetic distance between three autosomal avirulence loci, *vH3*, *vH5*, and *vH7H8*. Sixty-eight BC_1 males from this population were successfully mated and produced offspring (Table 1). The number of testcross families with an *H7H8*-virulent phenotype greatly outnumbered the families that had an *H7H8*-avirulent phenotype. This result was consistent with previous reports that avirulence to the combination of *H7* and *H8* is <100% penetrant (El Bouhssini *et al.* 2001), making it impossible to map *vH7H8* relative to the other avirulence loci in this experiment. Virulence and avirulence to both *H3* and *H5* did segregate in the expected 1:1 ratios. Moreover, among the 67 testcross families that infested both *H3*- and *H5*-resistant plants, 65 were of the parental type (29 virulent to both *H3* and *H5*, and 36 avirulent to both *H3* and *H5*). Thus, only 3 ± 2 cM appeared to separate avirulence genes *vH3* and *vH5*.

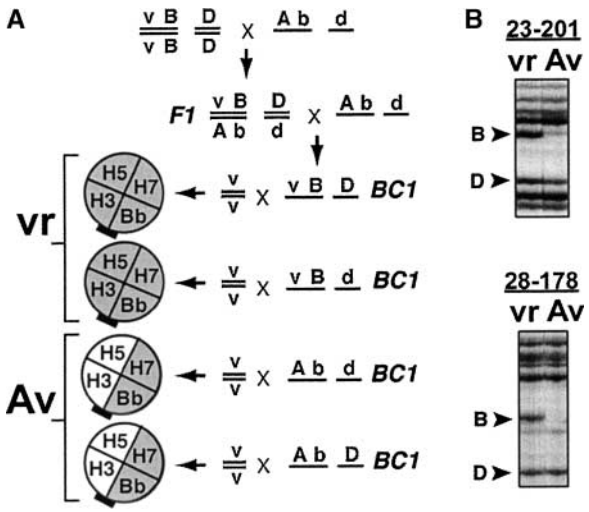

Figure 1.—Hessian fly matings and bulked segregant analysis for AFLP markers linked to autosomal avirulence genes in the Hessian fly. (A) The mating scheme. The avirulence (A) and virulence (v) alleles for a single avirulence gene are shown with bars representing chromosomes. Alleles of linked (B) and unlinked (D) AFLP loci are also shown. Only the transmitted chromosome sets (maternally derived) are shown for males. BC_1 males were testcrossed to virulent females, which were then caged separately on pots (circles) containing seedlings of four wheat cultivars carrying different resistance genes in four separate quadrants of the pot. (Bb represents "Blueboy" wheat, the susceptible check.) Shaded quadrants indicate susceptible plant reactions (virulent larvae). Unshaded quadrants indicate resistant plant reactions (avirulent larvae). For bulked segregant analysis, a virulent DNA pool (vr) and an avirulent DNA pool (Av) were prepared from the DNA of BC_1 males. (B) Segments of AFLP gels showing polymorphisms associated with the virulence-linked AFLP loci 23-201 and 28-178 (B) and the absence of polymorphisms associated with unlinked AFLP loci (D) as detected by the bulked segregant analysis of the virulent (vr) and avirulent (Av) DNA pools.

DNA polymorphisms linked to *vH5* and *vH3*: To identify molecular markers linked to *vH3* and *vH5*, bulked segregant analysis was performed using AFLP-PCR (Figure 1). A total of 1280 bands were observed in this analysis (data not shown). The number of polymorphic bands in phase with *H3* and *H5* virulence (eight) was 0.6% of the total. This value was only slightly greater than the frequency (0.2%) at which *vH13*-linked polymorphisms were observed in a previous investigation (Rider *et al.* 2002). Unexpectedly, however, 28 polymorphisms were also detected in phase with avirulence (data not shown), indicating that a considerable quantity of polymorphisms existed among individuals within the GP population. Six polymorphisms in phase with virulence were amplified from the virulent DNA pool and the DNA sequences of five of those polymorphic bands (22-130, 28-178, 23-201, 74-202, and 78-316) were determined (Figure 2). BLAST analyses (Altschul *et al.* 1997) indicated that no significant similarities existed between these sequences and others present in GenBank databases. Oligonucleotide primers designed to amplify two sequences (28-178 and 23-201) revealed codominant polymorphisms segregating among the BC_1 males in mapping population 1 (Figure 3). It was possible to amplify these markers among 64 of the 67 BC_1 males scored for virulence to both *H3* and *H5* (Table 2). Both molecular markers were genetically linked to *vH3* and *vH5* (Table 2). Although it was not possible

22-130 (BV079618)-
GAATTCACGA AGATGCACCG TTGGTCAAGG GGAATATAAC CAGGGCTAGA ATACGCGCAT GTACGGAGCG
TGTTTTTCCT GGAGCCAAAT GCTCCAGGGA GGAGCGCCCC TAACAGTAAC GCAGTGTTAA

28-178 (BV079619)-
GAATTCACCC ATTATTACAG AGGCTGATAA AGAAAGGTTT TTTTTTTGAT AAAAACTATA CAGAACACTT
TCATAGATAA GATCTT**CTGC GTTGAAAACA TGCTGT**TATT **GAACAA**TCGA GACTCCAGTT TCTATGTACG
AGAAATACGA GATGCTGAAG ACGTGATAAG GAAGTTAA

23-201 (BV079620)-
GAATTCACCC ATCAATCGTT CATTCGCACA CAAACACATA AACAGTAC**AA ACAATGC**TTT TGATACAGCA
AAAATGCGCC GAGGCAATAA ACAATTTCTT GATCTCAAAC GTGTCAAACA ACGATGGAAC ATAAAATGAA
TTTATTATGA TGATTTCGCC TTCGTTTACT CCATACTTCA AACACGTCGC GTCACTGTTA A

74-202 (BV079621)-
GAATTCTGTC GATACTTGTT TTTTAAATAT TTTTGATCAA ACGAACTCTC TCAAACTTTT CGAACGAACC
AAGGTATTTG GCCAATTCGT CTGTGCGAAA AGAGTGATTG AAAAAGGCCG GTAAGTTGAG ATAAATCATT
CAGTTTTATT GTTGTTGTGC TATGAATTTA TTTTGAAAAT GGGTAAAAAT CGATTTAGTT AA

78-316 (BV079622)-
GAATTCTGCA TTACCATTAT ATTTTGTAGA AAACGATGCC AACGAGAACC GTTTCTCTGT GGACGCAGCA
CATATTCGTA TTTTTGACGC AATCAGACTG CGTGTACAAT GTTCAGCGCA CATGTACACA AACACACCCA
AATCCATCAG TTGAATGATA GCAATGTGGG GTTTTCAATT GTGCTTTATT ATGTAGTTCA AAATGAAACC
ACAAATGAAT ACGCACCCGA ATGCACAATA CACCTGCCCC ATCGAAATGC ATTGCATAAT TACCGGTTTG
AATGATAGAA AGCTTCAGTG GCCGCACATA AGTTAA

L023 (BV079659)-
GCGATTGAAT TAGCGGCCGC GAATTCGCCC TTGATGTTAT ACACATGCAC CATTACCCAT ACCAATGGAA
TTTATTTATA CGAATTGGAT TGGCACACGA ATAGTTATTA TTATTATTAT TA**TTATTAT**C GTTTGTTTGG
TTTTGTGAAT GGATATGGGA TCGTTTTGTA TATATTTCCA ACAAGAGTAG CACCAACAAT AACAATAACA
ACAACAA**CAA** CGACGACCAC AACAATGCTC AATGTCCAAG TCTTATCGAA GGGCGAATTC GTTTAAACCT
GCAGGACTAG TCCCTTTAGT GAGGGTTAAT TCTGAGCTTG GCGTAATCAT GGTCATAGCT GTTTTTCCTG

L007 (BV079632)-
CTTTTTGGCC ACTTCATTAA GATTCGAGGC ATATTCACCG CCACAATTAC GGGCAATGGA TTTGATTCGA
GTATAAGCGT ATAATAGATA AACAGCTGTA TTTCCTTTGT CCTCCAACAT CTGAAATCCA AAAATGAAAA
AGAAAATAAT AATTGATCAT TCGTTTGCTT GGTCGTGAGT TCACCTACCC GATCGAATGA AAAAACGTAT
TCGTTGGTGC GATTCTTTGA AAGATCCGCA TACTTGATGC AACCATATGC AACCGATTCT TGAGCTTGTT
TCAATTCATC CGGCGTAAGA ACCTTATCAC GTTCTTTCTC CTTAAGTTTA TCCATGGCTC GGGTTAAACC
TTCGTCCAAT AAACTGGTTA ATTTCACTGT GTCACCTACA GGCAAACATT

L-009 (BV079623)-
GTTTTCGGCA ACGTTCCCAG AAGAAATTCG TAAGCTTGCA AGAAAATATC TGAAAGATAG CATCTTTTTG
AAAATTGGTT TGGTCGGTGG TGCTTGTGCA GATGTCACAC AAACTTTCTT CGAGGTCGAA AAAGTATTAA
GCAAGAAGCG TGACAAACTC ATGGAAATTT TAAATGAATC GGATCCAAAA GGTACGCATT AATCATATTT
AGTTCTTTCT AAAAATAGAA ATATAACATT TTTTTCATTC TTTCCTTTTT TTCTTTTTTT TTTTCATCAG
GAACAATGGT TTTTGTGGAA ACGAAAATTG CAGCAGACCG ATTGGCCGTA TTCCTCTCCG AGTCGGAGCA
CGCGACAACA TCAATTCATG GCGATCGGAA GCAATCACAA CGTGAAGAAG

FIGURE 2.—AFLP sequences discovered by bulked segregant analysis and STS sequences used to genetically position *vH3* and *vH5* in the Hessian fly genome. The GenBank accession numbers associated with each sequence are shown in parentheses. Sequences corresponding to primers designed to amplify these sequences are underlined. Using those primers, sequences 28-178, 23-201, and L023 functioned as codominant STS makers (Figure 3). The sequences in boldface type were absent in the alternative alleles.

to determine the order of these loci along the chromosome, the two avirulence loci appeared to be more closely linked to each other than to either of the DNA markers.

Physical positions of *vH3*- and *vH5*-linked polymorphisms: BAC clones containing 28-178 and 23-201 were identified in separate screenings of a Hessian fly BAC library and the positive BAC clones were used as probes in FISH to determine the physical positions of each marker on Hessian fly polytene chromosomes (Figure 4). Both markers hybridized to chromosome A2, indicating that *vH3*, *vH5*, and the two DNA markers are located on chromosome A2. However, 23-201 hybridized near the middle of the short arm of chromosome A2 whereas 28-178 hybridized near the middle of the long arm of chromosome A2. To test the possibility that the DNA sequence of each marker might be present at additional chromosomal positions, the genomic positions of each of five BAC clones containing 28-178 and two BAC clones containing 23-201 were determined. FISH was also performed on polytene chromosomes derived from both the L and GP populations to test for the existence of A2 chromosome rearrangements. In each experiment, the 28-178-containing BAC clones hybridized near the middle of the long arm of A2 and the 23-201-containing BAC clones hybridized near the middle of the short arm of A2 (data not shown). Thus, it appeared that although the DNA markers were genetically linked, they were physically separated by a distance of about one-half the length of the entire chromosome, including the centromere of chromosome A2.

Because of this unexpected observation, linkage between the molecular markers and virulence to Hessian fly resistance gene *H5* was retested using Hessian fly mapping population 2 (Table 1). This experiment included an independent marker (L023) that was devel-

TABLE 2

Recombination among *H3*- and *H5*-virulent and *H3*- and *H5*-avirulent BC_1 Hessian fly males and linked molecular markers

Population 1 BC_1 genotypes ($N = 64$)					
H3	*H5*	23-201	28-178	No.	Genetic distance (cM ± SE)
v	v	H	H	28	*vH3*–*vH5* = 3.1 ± 2
A	A	h	h	29	*vH3*–28-178 = 9.4 ± 4
A	v	H	H	1	*vH3*–23-201 = 6.3 ± 3
v	A	H	H	1	*vH5*–28-178 = 9.4 ± 4
A	A	H	H	3	*vH5*–23-201 = 6.3 ± 3
A	A	h	H	2	28-178–23-201 = 3.1 ± 2
All others				0	
Population 2 BC_1 genotypes ($N = 87$)					
H5	23-201	28-178	023	No.	Genetic distance (cM ± SE)
v	H	H	h	36	*vH5*–28-178 = 4.6 ± 2
A	h	h	H	45	*vH5*–23-201 = 3.4 ± 2
A	h	h	h	2	*vH5*–L023 = 6.9 ± 3
A	h	H	h	1	28-178–23-201 = 1.1 ± 1
A	H	H	h	3	28-178–L023 = 2.3 ± 2
All others				0	23-201–L023 = 3.4 ± 2

Hessian fly BC_1 males were either homozygous virulent (v) or heterozygous avirulent (A) for virulence and avirulence to Hessian fly resistance genes *H3* and *H5* and either homozygous (h) or heterozygous (H) at STS loci 28-130, 23-178, and L023.

oped on the basis of a DNA sequence of a Hessian fly genomic λ-clone (Figure 2) that had been previously identified on the short arm of chromosome A2 by *in situ* hybridization (Figure 4). Polymorphisms associated with L023 were in the opposite phase of those associated with 28-130 and 23-178 (Figure 3). A total of 102 BC_1 males of 110 tested were successfully scored for virulence to *H5* (Table 1). It was possible to score 87 of these for all three molecular markers (Table 2). Recombination among all four loci was detected among only 6 BC_1 males. This confirmed that *vH5* was genetically linked to the three A2 molecular markers. However, the order of the markers suggested by this experiment [*vH5*-(23-201)-(28-178)-L023] differed from the physical positions of the molecular markers on the chromosome [L023-(23-201)-centromere-(28-178)]. Greater recombination was observed between L023 and 23-201 (markers on the short arm of A2) than between L023 and 28-178 or 23-201 and 28-178 (markers on opposite arms of A2). Thus, it appeared that a low coefficient of exchange in the proximal region of chromosome A2 had interfered with our attempt to position *vH3* and *vH5* along that chromosome.

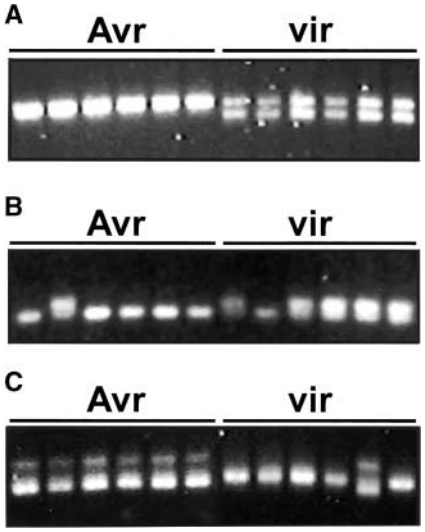

FIGURE 3.—Examples of the polymorphisms associated with the STS markers used to genetically map *vH3* and *vH5* in the Hessian fly genome. The amplicons of markers 28-178 (A), 23-201 (B), and L023 (C) are shown associated with the DNA of six different *H5*-avirulent (Avr) and six different *H5*-virulent (vir) BC_1 male Hessian flies. Note that recombinant types are visible among the individuals shown for markers 23-201 and L023.

AFLP-based genetic map of the Hessian fly genome: To test the possibility of recombination suppression further, we developed an AFLP-based genetic map using a small ($N = 55$) female family derived from a cross between Hessian flies avirulent to the *H3* resistance gene. To help establish linkage group-chromosome correlations, six STS markers previously positioned on three of the polytene chromosomes by *in situ* hybridization were included with the AFLP markers in the construction of the map (Table 3, Figures 2 and 5). STS L009 marked the tip of the short arm of chromosome

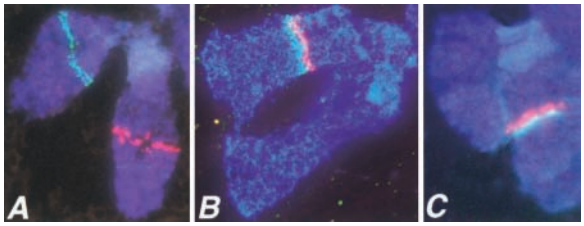

FIGURE 4.—Chromosomal locations of *vH3*- and *vH5*-linked markers. (A) *In situ* hybridization of BAC clones containing markers 23-201 and 28-178. Marker 23-201 (green fluorescence) hybridized to the short arm of A2 and marker 28-178 (red fluorescence) hybridized to the long arm of chromosome A2. Note that the homologous chromosome arms have failed to pair around those positions of the chromosome. (B) *In situ* hybridization of BAC clones containing markers 23-201 and L023. The position of L023 (green fluorescence) was just distal to that of 23-201 (red fluorescence) on the short arm of chromosome A2. (C) *In situ* hybridization of BAC clones containing markers 8309 and 28-178. The position of marker 28-178 (green fluorescence) was just distal to that of 8309 (red fluorescence) on the long arm of chromosome A2.

A1. STSs L023 and L007 marked the short arm of chromosome A2, and 28-178 marked the long arm of chromosome A2 (marker 23-201 was not polymorphic in this mapping population). STSs 23-134 and 22-124 marked the tip of the short arm of X2 and STS G15-1 marked the X2 centromere. The sequences associated with markers G15-1 (STUART *et al.* 1998) and 23-134 and 22-124 (RIDER *et al.* 2002) have been published elsewhere.

A total of 183 polymorphic AFLP bands were observed. On the basis of the quality of the bands, 108 of these were selected for further analysis. Of these 108, 7 were eliminated because their segregation deviated from the expected 1:1 ($\chi^2 > 3.84$, 1 d.f., $\alpha = 0.05$). The remaining 101 AFLP and six STS polymorphisms were used to develop a map that consisted of 69 genetic loci on four linkage groups (Figure 6). There was complete cosegregation among some of the markers so that 15 loci were identified multiple times. The entire map covered 443.4 cM and the loci on the map were an average of 6.9 ± 4.8 cM apart.

The linkage map appeared to be a reasonable representation of the Hessian fly genome, and on the basis of the physical positions of markers previously positioned on the polytene chromosomes, each linkage group appeared to correspond to a different Hessian fly chromosome (Figure 6). L009, which had been positioned near the telomere of the short arm of chromosome A1, was located at one end of the first linkage group (LG A1). Chromosome A2 markers L007, L023, and 28-178 were all located on LG A2. Chromosome X2 markers G15-1, 23-234, and 22-123 were present on LG X2. Thus, it appeared that LG A1 corresponded to chromosome A1, LG A2 corresponded to chromosome A2, LG X1 corresponded to chromosome X1, and LG X2 corresponded to chromosome X2. Furthermore, the amount of recombination estimated for each linkage group was closely correlated with the relative lengths of the corresponding chromosomes (Table 4). In addition, the percentage of the S genome associated with the autosomes and X chromosomes was strikingly similar to the percentages associated with the numbers of recombination units along the autosomes and the X chromosomes, respectively (Table 4).

To test the correspondence between the linkage groups and the chromosomes further, 35 of the AFLPs on the map were cloned and sequenced in an effort to develop probes for FISH (Table 3). Twenty-eight of these AFLP sequences were used to probe the BAC library and 26 were hybridized to at least one BAC. Four AFLP sequences, each hybridizing to >50 BAC clones in the library, were clearly repetitive. FISH was used to position 20 of the AFLPs containing BAC clones on the polytene chromosomes of the Hessian fly (Figure 5). Only 6 clones failed to hybridize at the predicted positions (Figure 6). Two of those (6502 and 2704) contained repetitive sequence and hybridized to the centromeres of the polytene chromosomes. The nonrepetitive markers that failed to hybridize in expected positions were all associated with LG A1 and LG X1. When the AFLP and STS markers were combined and the repetitive sequences were ignored, 23 of the 27 loci tested by FISH (85%) were located in regions of the chromosomes predicted by the genetic map. Thus, the genetic map appeared to be a reasonable representation of all the Hessian fly chromosomes and was anchored particularly well with respect to Hessian fly chromosomes A2 and X2.

Consistent with a low coefficient of exchange in the proximal region of chromosome A2, eight AFLPs (8% of the total number of AFLPs mapped) cosegregated with L023 at position 86.5 on LG A2 (Figure 6). In addition, a cluster of five loci, corresponding to 16% of the total number of markers on the map, encompassed L023 from position 84.6 to 94.0 on LG A2 (Figure 6). Marker 28-178 was located in that cluster, cosegregating with AFLP 8309 at position 88.3, only 1.8 cM from L023. Furthermore, the cytological position of 8309 was determined by FISH to be just distal of the position of 28-178 in the proximal region of chromosome A2 (Figure 4). Thus, ~50% of Hessian fly chromosome A2, extending from the cytological position of marker L023 across the centromere to the cytological position of 28-178, appeared to correspond to <2% of the genetic length of the chromosome.

DISCUSSION

Bulked segregant analysis identified AFLPs linked to avirulence genes *vH3* and *vH5*. The positions of these markers on polytene chromosome A2 of the Hessian fly indicated that both of these avirulence genes are located on chromosome A2. However, approximately one-half the length of the chromosome and the centro-

TABLE 3

AFLP and STS sequences used to screen the Hessian fly BAC library and the corresponding BAC clones used as probes in FISH experiments with Hessian fly polytene chromosomes

AFLP/STS marker[a]	Genetic position	GenBank accession no.	Clones[b] (no. of BACs)	Physical position
STS L009 (*Dm-vasa*, NM057434)	LG 1-0.0	BV079623	L (1)	A1S-T
AFLP 1406	LG 1-34.5	BV079624	5j22 (2)	A2L
AFLP 1408	LG 1-45.9	BV079625	5o5 (3)	X1L/A2L
AFLP 1410	LG 1-91.3	BV079626	38d24 (5)	X1L-T
AFLP 2503	LG 1-125.3	BV079627	5a14 (12)	A1L
AFLP 6707	LG 1-129.2	BV079628	14c3 (1)	A1L
AFLP 2709	LG 1-129.2	BV079629	12f21 (4)	A1L
AFLP 7207	LG 2-0.0	BV079630	39c14 (>50)	A2S-T
AFLP 2512	LG 2-60.6	BV079631	25j8 (1)	A2S
STS L007 (*Dm-ARGRS*, AE003500)	LG 2-71.7	BV079632	L	A2S
STS L023	LG 2-86.5	BV079659	L (1)	A2S
AFLP 8307	LG 2-86.5	BV079633	(0)	Unknown
AFLP 6503	LG 2-86.5	BV079634	(>50)	Unknown
AFLP 8309	LG 2-88.3	BV079635	36c7 (2)	A2L
AFLP 7701	LG 2-92.2	BV079636	NP	Unknown
AFLP 2704	LG 2-94.0	BV079637	17b19 (>50)	Centromeric
AFLP 1308	LG 2-94.0	BV079638	(0)	Unknown
AFLP 2706	LG 2-114.1	BV079639	9k9 (9)	A2L
AFLP 2510	LG 2-123.8	BV079640	NP	Unknown
AFLP 7302 (*Dm*-AAF55827)	LG 3-0.0	BV079642	9j7 (1)	X1S
AFLP 2710	LG 3-10.4	BV079643	5a3 (3)	X1S
AFLP 7708	LG 3-37.5	BV079644	(>50)	NP
AFLP 2507	LG 3-50.8	BV079645	7h8 (2)	NP
AFLP 1307 (*Ag*-XM_308836.1)	LG 3-64.7	BV079646	22c11 (5)	X1L
AFLP 1303	LG 3-64.7	BV079647	2n17 (6)	X1L
AFLP 8308	LG 3-66.5	BV079648	16d20 (1)	A1L
AFLP 6502	LG 3-76.1	BV079649	4g18 (6)	Centromeric
AFLP 6703	LG 3-83.6	BV079650	NP	Unknown
AFLP 1404	LG 4-0.0	BV079651	10b5 (10)	Unknown
STS 23-134	LG 4-8.7	AF424883	L, 37L3 (4)	X2S
AFLP 1309	LG 4-13.4	AF424882	15o2 (5)	X2S
STS 22-124	LG 4-15.5	AF424881	L, 5j15 (3)	X2S
AFLP 7209	LG 4-21.0	BV079660	3d10 (2)	X2S
AFLP 6505	LG 4-45.9	BV079652	NP	Unknown
AFLP 8205	LG 4-45.9	BV079653	NP	Unknown
STS G15-1	LG 4-51.5	AF051559	L	Centromeric
AFLP 7817	LG 4-51.5	BV079654	NP	Unknown
AFLP 2712	LG 4-51.5	BV079655	NP	Unknown
AFLP 6504	LG 4-55.2	BV079656	6p9 (1)	NP
AFLP 2505	LG 4-72.4	BV079657	31h18 (1)	X2L
AFLP 2205	LG 4-85.6	BV079658	35g17 (1)	Unknown

[a] Notes in parentheses indicate significant BLASTX scores (≤E-10) and the accession numbers of the most significant sequence similarity. *Dm*, *Drosophila melanogaster*; *Ag*, *Anopheles gambiae*.

[b] L indicates genomic λ-clone; all others are BAC clones.

mere separated the positions of the AFLPs on chromosome A2. This was the first evidence of the existence of severe recombination suppression in the Hessian fly genome. These observations were confirmed in experiments with two additional mapping populations and the construction of the first genetic map of the Hessian fly genome. In all three mapping populations, markers physically positioned in the middle of opposite arms of chromosome A2 showed <3% recombination. Further, this low coefficient of genetic exchange was independent of virulence to *H5* as it was evident in two *H5*-avirulent strains. It was also independent of the sex of the mapping population since it was observed in both male mapping populations 1 and 2 and the female mapping population used to construct the genetic map.

Variation in recombination rates across eukaryotic chromosomes has been observed in a variety of eukaryotic species (Tanksley *et al.* 1992; Nicolas 1998; Yu *et al.* 2001; Boyko *et al.* 2002), and reduced recombination near the centromere is not unusual (Roberts 1965;

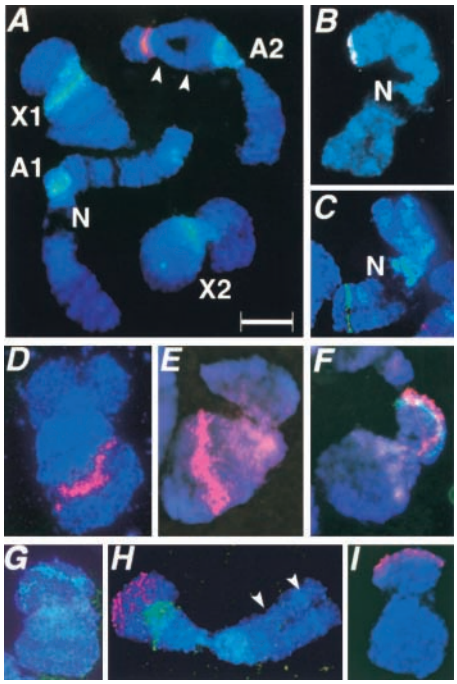

Figure 5.—*In situ* hybridizations of BAC clones containing AFLP markers to the Hessian fly polytene chromosomes. (A) All four chromosomes are visible and the position of the nucleolus (N) on chromosome A1 is indicated. Arrowheads indicate the extent to which the homologous chromosomes failed to pair along the short arm of polytene chromosome A2. AFLP 2704 (green fluorescence) hybridized near the centromeres of all four chromosomes. AFLP 2706 (red fluorescence) hybridized near the tip of the long arm of chromosome A2. (B) AFLP 2701 (green fluorescence) near the telomere on the short arm of chromosome A1. (C) AFLP 2709 (green fluorescence) near the tip of the long arm of chromosome A1. (D) AFLP 1307 (red fluorescence) in the middle of the long arm of chromosome X1. (E) AFLP 2505 (red fluorescence) near the middle of the long arm of chromosome X2. (F) Markers 22-124 (green fluorescence) and 23-134 (red fluorescence) near the telomere of the short arm of chromosome X2. (G) AFLP 7302 (green fluorescence) near the tip of the short arm of chromosome X1. (H) AFLP 2512 (red fluorescence) and marker L023 (green fluorescence) on the short arm of chromosome A2. Arrowheads indicate the extent to which the homologous chromosomes failed to pair along the long arm of polytene chromosome A2. (I) AFLP 1309 near the telomere on the short arm of chromosome X2.

Tanksley *et al.* 1992; Boyko *et al.* 2002). Nonetheless, the most interesting parallel to the low incidence of recombination over the proximal region of the Hessian fly chromosome A2 is probably that of the *Drosophila melanogaster* chromosome 3 (Green 1975; Sinclair 1975; Denell and Keppy 1979). The proximal region of chromosome 3 makes up 20% of its mitotic length but only 1% of its genetic length (Sinclair 1975). In comparison, the centromeric heterochromatin of Hessian fly chomosome A2 makes up ~30% of the mitotic chromosome (Stuart and Hatchett 1988b) and, according to the present study, accounts for no more than 3% of its total genetic length. Further, negative chromosome interference, a normal characteristic of the proximal region of Drosophila chromosome 3 (Green 1975; Sinclair 1975; Denell and Keppy 1979), may also be characteristic of the proximal region of Hessian fly chromosome A2. We observed among the 87 individuals segregating for markers flanking the chromosome A2 centromere (L023, 23-201, and 28-178) in population 2 (Table 2) that the expected frequency of double recombination between the three DNA markers was 0.0004. Thus, we might have expected to observe one double recombinant among 2500 individuals. We actually observed one among only 67 individuals. Taken together, these observations suggest that the recombination suppression we observed in the proximal region of Hessian fly chromosome A2 was not the consequence of rearrangements, but a normal characteristic of the chromosome. However, it is interesting to note that the homologs often fail to pair near the middle of both arms of polytene chromosome A2 (Figures 4 and 5). Perhaps this reflects the existence of the type of interruptions in homology between chromatids that has been proposed to disturb synaptonemal complex formation and cause negative chromatid interference (Sybenga 1996).

We noted that the proximal region of Hessian fly chromosome A2 was associated with a 9.4-cM region on the genetic map between positions 84.6 and 94.0 cM on LG A2 that contained 16% of the markers used to construct the genetic map. Four other genomic regions were associated with an abundance of markers and thus may also experience recombination suppression: The first region extends from 125.3 to 130.8 cM on LG A1 (Figure 6). This genetic region was associated with 13 markers and apparently corresponds to the genomic region near the tip of the long arm of chromosome A1. The second genetic position extends from 91.3 to 93.2 cM on LG A1. It was also associated with 13 markers. Although its corresponding chromosomal position was not determined, it seems reasonable to speculate that these markers may correspond to the centromeric region of chromosome A1. The third genetic region with an abundance of markers extended from 62.8 to 66.5 cM on LG X1. This region was associated with 6 markers that corresponded to the middle of the long arm of chromosome X1. The fourth genetic position is centered at 51.5 cM on LG X2. This position was associated with 6 markers and corresponded to the centromeric region of chromosome X2.

Our long-term goal is to clone and characterize Hessian fly avirulence genes. Toward that goal, previous efforts focused on the use of bulked segregant analysis as the most efficient method (Stuart *et al.* 1998; Schulte *et al.* 1999; Rider *et al.* 2002). However, the present analysis demonstrates the utility of having a physically anchored genetic map as an aid in this process. Since avirulence gene discovery has been managed primarily through map-based cloning methods (Leach

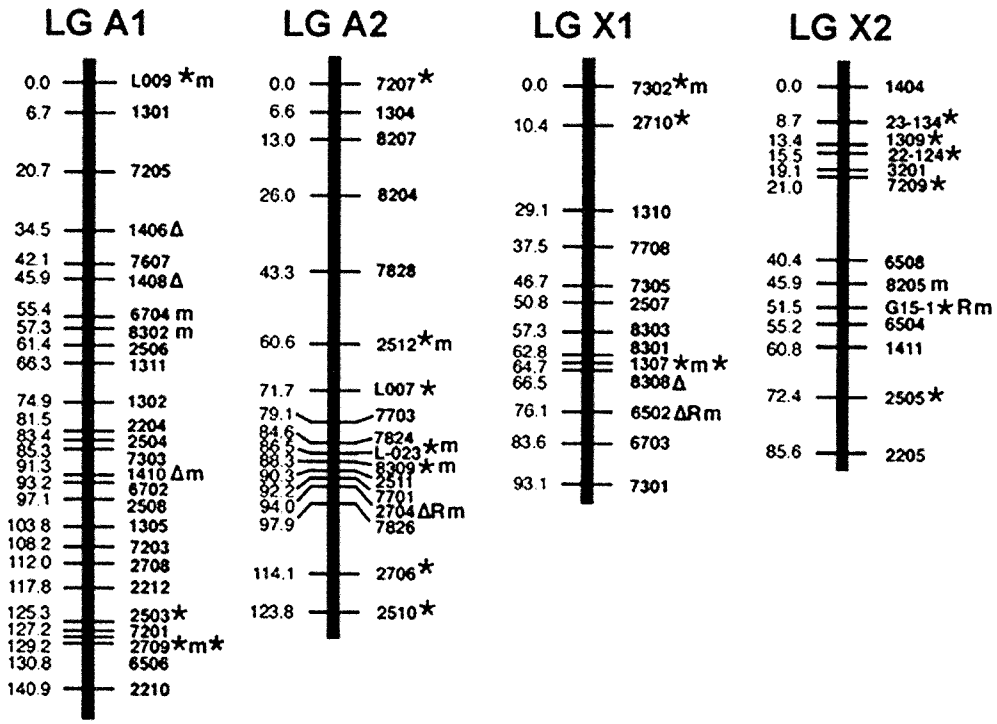

FIGURE 6.—AFLP-based linkage map of the Hessian fly genome. The map distances (Kosambi centimorgans) are shown on the left and the marked loci are shown on the right of each of the four linkage groups. Multiple markers identified loci marked "m." Twenty-eight markers at 24 loci were used to screen a BAC library for clones containing the markers. Loci marked by an asterisk were associated with BAC clones that hybridized at a corresponding physical position on the Hessian fly polytene chromosomes. Loci marked by a triangle were associated with BAC clones that failed to hybridize to a corresponding physical position on the polytene chromosomes. Loci marked by an "R" were associated with AFLPs that either identified >50 BAC clones in a library screen or hybridized to multiple positions on the polytene chromosomes.

and WHITE 1996; BONAS and ACKERVEKEN 1999; WHITE *et al.* 2000), the discovery of crossing-over suppression is significant. The Hessian fly genetic map will permit us to identify and focus on avirulence genes in genomic locations in which recombination frequency is greater. With regard to this consideration, it is interesting to note that in comparison with the investigation that mapped *vH13* (RIDER *et al.* 2002), the number of polymorphisms discovered by bulked segregation analysis that were out of phase with virulence was 10 times greater. This might be explained, at least in part, by the greater frequency of recombination evident near the position of *vH13* on the genetic map, the region marked by STS markers 22-124 and 23-134 near the end of LG 4 and corresponding to the tip of the short arm of chromosome X2 (Figures 5 and 6).

To anchor the genetic map to the Hessian fly polytene chromosomes, we physically positioned AFLPs and STS markers on the polytene chromosomes of the Hessian fly salivary gland. The correlation was imperfect. This was expected because the processes of both creating and testing the map involved several steps in which errors could occur. First, the sample size was small and likely pushed the limits of MAPMAKER to build an

TABLE 4

Comparisons of genetic distance with relative Hessian fly chromosome length and genome size

	Chromosome			
	A1	A2	X1	X2
% relative length (mitotic)[a]	30.1	23.6	25.0	21.6
% relative length (polytene)[a]	36.5	27.0	19.0	17.5
% genetic distance	31.7	27.9	20.9	19.3

	Chromosome	
	A1 + A2	X1 + X2
Chromosome lengths (% of total)[b]	86 Mb (53.8)	74 Mb (46.6)
% genetic distance	59.7	40.3

[a] As determined by STUART and HATCHETT (1988b).
[b] J. S. JOHNSTON, personal communication.

accurate map with the marker data. Second, cloning and sequencing the AFLP bands that were extracted from the gels often resulted in more than one DNA sequence associated with each band. Thus, the wrong sequence may have occasionally been used as a probe in the identification of BAC clones that were used to position the markers on the chromosomes. Third, if the marker contained a motif common to more than one location in the genome, BAC clones derived from the wrong chromosomal position may have been used to perform FISH. Nonetheless, the present work has resulted in a scaffold of physically and genetically anchored BAC clones that will be useful in more detailed investigations of the Hessian fly genome.

Characterization of the BAC library used in this investigation has not been published previously. Developed in collaboration with Research Genetics (Invitrogen), it consists of 18,482 clones that, on average, have 55-kb inserts (data not shown). The S genome of the Hessian fly contains 160 Mb of DNA (J. S. Johnston, personal communication), giving this library an estimated sixfold genomic coverage. If only nonrepetitive clones are considered, screening the library with AFLP-derived fragments identified only an average of 3.3 ± 3.1 clones/screen. Two AFLPs failed to identify a clone in the BAC library (Table 3). Therefore, although it contributed greatly to the development of a physical-genetic map of the Hessian fly, additional BAC libraries will be desirable for future investigations.

The facility for genetic analysis in the Hessian fly, its small genome size (160 Mb), and its pest status make it an attractive model for member species of the family Cecidomyiidae (gall midges; Harris *et al.* 2003). Together with the blackflies, sandflies, midges, mosquitoes, and fungus gnats, the gall midges are classified in the suborder Nematocera in the order Diptera (Arnett 2000). The Cecidomyiidae is one of the larger families in the Diptera, composed of ~5000 described species (Harris *et al.* 2003). The Hessian fly is a member of the subfamily Cecidomyiinae, the largest and youngest group of gall midges, which includes a relatively large number of important plant pests such as the Asian rice gall midge (*Orseolia orzyae*), the African rice gall midge (*Orseolia oryzivora*), the sorghum midge (*Contarinia sorghicola*), the orange blossom wheat midge (*Sitodiplosis mosellana*), the barley stem gall midge (*Mayetiola hordei*), and the sunflower midge (*Contarinia schulzi*), to name just a few. Interestingly, within the Cecidomyiiae, gross genomic organization has been remarkably conserved (White 1950; Stuart and Hatchett 1988a; Sahu *et al.* 1996). All species so far examined contain both an S genome and germ-line-limited E chromomosomes. Further, except for a single species referred to as *Phytophaga celtiphyllia* by White (1950), the S genome is composed of two autosomes and two X chromosomes. Most species also appear to reproduce via families that are either monogenous or highly biased toward one sex (Barnes 1931; Baxendale and Teetes 1981; Stuart and Hatchett 1988a). Further, the radiation of this group of midges with the evolution of flowering plants is an intriguing example of diversification, specialization, coevolution, and speciation (Gagne 1989). Approximately 60% of the Cecidomyiinae possess the ability to evoke a specific growth response in host plant tissue that leads to the formation of a gall. These gall midges are generally monophagous or restricted to living on a limited group of related plant species.

The Asian rice-gall midge interaction has also clearly demonstrated that the Hessian fly is not the only gall midge with a gene-for-gene relationship with its host plant (Nair *et al.* 1995; Mohan *et al.* 1997; Behura *et al.* 2001; Bentur *et al.* 2003). Such relationships are likely present among other insect plant parasites such as aphids (Rossi *et al.* 1998). However, the genetic tractability of the Hessian fly makes it particularly well suited to an analysis of the mechanisms underlying insect-plant gall formation and gene-for-gene relationships. Among the tools that are likely necessary for such analyses are the ability to induce mutations in the Hessian fly, which is possible although complicated by its life cycle (Stuart *et al.* 1997), and the ability to genetically transform this insect, a technology that is still lacking. Until now, a useful genetic map of the Hessian fly genome was also missing. Thus, the physically anchored AFLP-based genetic map of the Hessian fly generated by this investigation constitutes the first genetic map constructed of any gall midge. We expect that this resource, combined with the presence of polytene chromosomes in the salivary glands of most gall midge species, will permit more detailed comparisons of genomic organization among gall midge species and aid a thorough analysis of the mechanisms underlying chromosome imprinting and sex determination in the Cecidomyiidae.

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